

## THE DEMONSTRATION OF PHOSPHATASE IN NEISSERIA<sup>1</sup>

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Although the study of bacterial enzymes has received much attention during the last few years, it was not until recently that the presence of phosphatase in microorganisms was established. The first evidence that this enzyme occurred among bacteria was the demonstration of the ability of *Escherichia coli* to hydrolyze hexosephosphate (Manning, 1927). Several years later, Boivin and Mesrobian (1933) observed that inorganic phosphate was liberated from a variety of other substrates on hydrolysis by this organism at characteristic pH optima. In 1938, Pett and Wynne measured the activity of phosphatase in a heterogeneous group of saprophytic bacteria and noted, in addition, an optimal pH for each organism. Pett (1939) suggested that the presence of phosphatase might prove valuable in the classification of bacteria.

A recent study by Leahy, Sandholzer and Woodside (1939) showed that, of a considerable number of strains of gram-negative bacilli belonging to the family *Enterobacteriaceae*, every one tested hydrolyzed disodium-phenyl-phosphate. By employing this substrate, quantitative information was obtained with the use of smaller quantities of bacterial cells than had heretofore been possible. The optimal pH varied among the different genera and even among strains of the same species. The present report, a continuation of this study, deals with the demonstration of

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phosphatase in the *Neisseria*, especially in *Neisseria gonorrhoeae*. It was undertaken with the hope that the new approach offered by quantitative biochemical methods might yield data pertinent to a better understanding of the relationships in this particular group where other methods of approach have not been very fruitful.

#### METHODS

##### *Preparation of the bacterial cells*

For the present investigation, 16 strains of *Neisseria* were employed. Of these strains, 10 were *Neisseria gonorrhoeae*, 2 *Neisseria intracellularis*, 2 *Neisseria catarrhalis*, and 2 *Neisseria sicca*. With the exception of preparations GN I and GN II, each strain was grown for 6 or 7 days at 37°C. in Douglas's broth containing 5 per cent of ascitic fluid. The cells were removed by centrifugation, washed once in a 0.85 per cent solution of NaCl (physiological saline solution), "lyophilized" by the technic of Flosdorf and Mudd (1935), placed in stoppered test tubes, and stored at 5°C. until measurements were made. The preparations of dried cells from strains GN I and GN II were obtained from 6-day-old veal-infusion-broth cultures.<sup>2</sup>

##### *Preparation of the substrate solutions*

The buffered substrate solutions employed for the measurement of pH optima were prepared as described by Leahy, Sandholzer and Woodside (1939). The 0.005 *M* disodium-phenyl-phosphate was buffered with a 0.05 *M* phthalate or veronal buffer at each pH unit from 4.0 to 9.0. After adjustment to the desired pH value had been made, it was checked by the glass-electrode method before the solution was used.

##### *Measurement of optimal pH*

The phosphatase activity of the "lyophilized" organisms was measured by the method of Leahy, Sandholzer and Woodside (1939). In brief, the procedure consisted in adding a known

<sup>2</sup> Supplied by Parke, Davis and Company, Detroit, Michigan.

weight (from 2 to 5 mgm.) of "lyophilized" cells suspended in distilled water to a series of tubes containing buffered substrate solutions at each pH unit from 4.0 to 9.0. The mixtures were incubated for 24 hours at 37°C.,  $\pm 0.02^\circ\text{C}$ ., and the degree of hydrolysis was measured from the amount of phenol produced. Quantitative colorimetric estimation of the phenol was made by the use of Gibbs's reagent (2,6-dibromoquinonechloroimide), which is capable of measuring 0.1 gamma of phenol in 10 ml. The activity of the various preparations was expressed in gammas of phenol liberated per milligram of "lyophilized" cells in 24 hours at 37°C. after a small correction, as indicated by the controls, had been made. In every instance, the pH of maximal activity was determined by plotting curves of the phenol values at each pH. Duplicate runs with different preparations of the same strain, and repetition of measurements, both in the absence and in the presence of magnesium ions, gave optima agreeing within  $\pm 0.2$  pH unit.

#### RESULTS

The optimal pH values for the organisms of the genus *Neisseria* selected for study, are shown in table 1. Without exception, all of the strains examined possessed phosphatase activity. The maximal activity of the different preparations varied widely. Two strains of *Neisseria gonorrhoeae*, 21 and 24, were the most active of all the *Neisseria* examined, while the 2 strains of *Neisseria sicca* displayed the weakest activity.

Many of the strains manifested sharp pH optima, but these optima varied considerably among the several preparations measured. Of the 16 strains of *Neisseria*, 5 strains of *Neisseria gonorrhoeae* had sharp optima within a narrow range from pH 4.7 to 4.9, 9 from pH 6.1 to 6.4, and 1 at pH 5.2. On the other hand, another strain of *Neisseria gonorrhoeae* possessed a comparatively broad pH range of activity, from pH 6.8 to 7.4.

An experiment to determine the effect of storage upon the activity of a single preparation (GN I) is illustrated in figure 1. Storage for a period of 100 days at 15 mm. pressure at room temperature decreased the activity by 32 per cent. Another ex-

TABLE 1

The maximal activity of phosphatase at optimal pH in various strains of *Neisseria*

STRAIN NUMBER	ORGANISM	SOURCE	MAXIMAL ACTIVITY AT OPTIMAL pH	pH OF MAXIMAL ACTIVITY
6	<i>N. gonorrhoeae</i>	Joint	35.3	4.7
10A	<i>N. gonorrhoeae</i>	Urine	50.3	4.8
16A	<i>N. gonorrhoeae</i>	Urine	40.2	4.8
2	<i>N. gonorrhoeae</i>	Eye	30.8	4.9
16	<i>N. gonorrhoeae</i>	Urine	43.0	4.9
24	<i>N. gonorrhoeae</i>	Cervix	60.5	5.2
21	<i>N. gonorrhoeae</i>	Joint	61.3	6.1
GN I	<i>N. gonorrhoeae</i>	Urethra	41.2	6.1
69	<i>N. gonorrhoeae</i>	Urine	29.5	6.3
GN II	<i>N. gonorrhoeae</i>	Urethra	47.6	7.2*
38	<i>N. intracellularis</i>	Sp. fluid	14.6	6.2
18	<i>N. intracellularis</i>	Sp. fluid	58.0	6.4
60	<i>N. catarrhalis</i>	Urine	21.3	6.5
26	<i>N. catarrhalis</i>	Urine	21.0	6.7
34	<i>N. sicca</i>	Urine	12.7	6.1
41	<i>N. sicca</i>	Urine	12.9	6.2

\* The range of optimal pH was from pH 6.8 to 7.4.

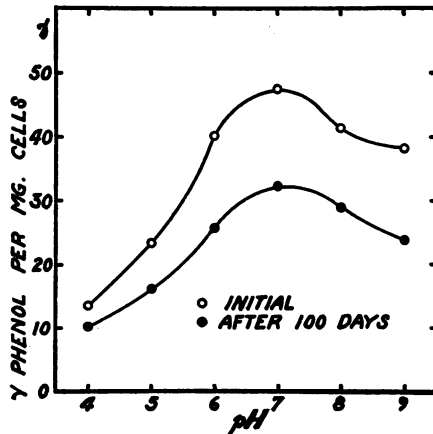


FIG. 1. pH-ACTIVITY CURVES OF NEISSERIA GONORRHOEAE (GNII) BEFORE AND AFTER STORAGE FOR 100 DAYS AT 15 MM. PRESSURE AT ROOM TEMPERATURE

periment to determine the effect of magnesium ions upon the same preparation (fig. 2) showed that a 0.001 *M* concentration of these ions stimulated the activity by 11 per cent. Strikingly, in each experiment, the pH of maximal activity remained unchanged.

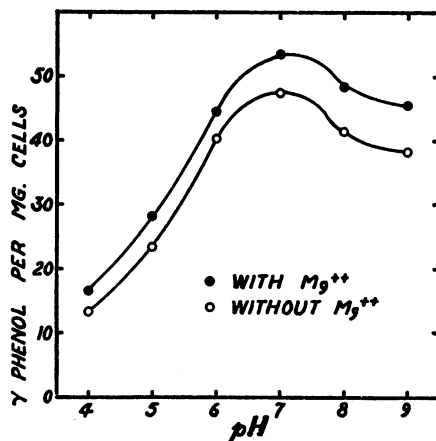


FIG. 2. pH-ACTIVITY CURVES OF NEISSERIA GONORRHOEAE (GNII) WITH AND WITHOUT ADDED 0.001 *M*. MAGNESIUM IONS

#### DISCUSSION

The use of a sensitive substrate (disodium-phenyl-phosphate) and the controlling of conditions affecting hydrolysis have made it possible to measure the activity of phosphatase by a method in which the test-organism was the only variable. Application of this method to the genus *Neisseria* disclosed that both the optimal pH and the maximal activity varied markedly among the different organisms examined. Of these characteristics, the variation of the optimal pH is considered to be the more significant for the following reasons. First, although repeated measurements on duplicate preparations have shown a decreased activity upon storage (fig. 1), the pH of maximal activity remained constant. Second, measurements made in the presence of added magnesium ions (fig. 2) have shown an increased activity, while again the optimal pH failed to show a significant change.

The 5 strains of *Neisseria gonorrhoeae* which had optima in the range of pH 4.7 to 4.9 possessed the most acid optima of all the microorganisms yet encountered in our studies using disodium-phenyl-phosphate. The *Neisseria* as a whole, furthermore, were found to have maximal activities far below those of the *Enterobacteriaceae*, as described in a previous study.

## SUMMARY

Phosphatase has been demonstrated for the first time in *Neisseria gonorrhoeae*, *Neisseria intracellularis*, *Neisseria catarhalis*, and *Neisseria sicca*. The optimal activity occurred at pH values ranging from pH 4.7 to 7.2. Each strain possessed a single characteristic optimum.

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